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The Function of the Madreporite in Body Fluid Volume Maintenance by an Intertidal Starfish, *Pisaster ochraceus*

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Abstract. The madreporite has been viewed as superfluous and unnecessary because starfish can keep their tube feet inflated by osmotic mechanisms alone. Recent evidence has suggested, however, that the madreporite may be significant in the replenishment of general body fluid. This hypothesis has been tested. The madreporite openings of an intertidal starfish, *Pisaster ochraceus*, were obstructed with cement, and the animals were used in controlled experiments to compare weight (volume) changes under stable conditions, in response to air drying and recovery, and during adaptations to hyper- and hypoosmotic environments. Over a period of days, normal animals showed positive and negative volume fluctuations of up to about 20% (in part related to posture). Animals with obstructed madreporites generally did not gain weight and were significantly less able to maintain body volume or recover from fluid losses resulting from the stresses applied. The madreporite seemed to contribute little to the initial osmotic responses, but it did participate in subsequent volume readjustments in a hyperosmotic medium that had induced fluid losses. Obstruction of the madreporite did not impede tube foot activity, but may have caused some diversion of general body fluid to the ambulacral system. Rates of seawater uptake through the madreporite of 2.2–2.6 $\mu l\ g^{-1}\ h^{-1}$ were calculated from observed maximum mean differences in weight changes.

Introduction

A sieve-like structure known as the madreporite is conspicuously located on the aboral disk of most starfish. The madreporite contains pores that mostly open into a reinforced ciliated duct, the stone canal, which in turn leads to the oral side of the body and a system of canals extending to the tube feet (Ferguson and Walker, 1991). Although many scientists have assumed that the function of the madreporite is to admit seawater to the tube feet, thus supporting their operation as hydraulic devices, Hyman (1955) pointed out that there was little reliable evidence for that assumption, and that any fluid loss and replacement from this "water vascular system" must be slight. Further, Robertson (1949) showed that the fluid found within the tube feet of *Marthasterias glacialis* was different from seawater, being especially elevated in potassium ion concentration. These observations led Binyon (1961, 1962, 1964, 1966, 1976a, b, 1979, 1980, 1984) to examine the dependence of tube feet on madreporitic water inflow much more closely. He found that in *Asterias rubens*, these appendages continued to function quite well, even if all the water passages were plugged, the madreporite removed, or the arms separated entirely from the disk. After measuring pressure levels and permeability factors, he concluded that tube foot activity must be sustained by the osmotic influx of water directly through their walls. Additional evidence of the importance of osmotic factors came from Prusch and Whoriskey (1976) and Prusch (1977), who not only directly measured the osmotic elevations of tube foot fluid in *Asterias forbesi*, but also demonstrated in these tube feet an active transport system for potassium ions that is capable of sustaining the osmotic differences. More recently, Ferguson (1990a) has provided data on 14 species of starfish, all showing osmotic elevation of ambulacral fluids.

These studies have convinced many modern workers that the madreporite cannot be important to the normal operation of tube feet. Disconcertingly, there are few data

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to show that the structure does anything else either. A number of functions have been suggested, *e.g.*, that it is some kind of sense organ, a secretory or excretory device, an emergency relief valve, or a supplementary pump that functions only in periods of stress, but evidence for these kinds of functions is trivial (Binyon, 1964; Nichols, 1966; Prusch, 1977).

Noting this confusion, Ferguson (1987) initiated a series of experiments on *Echinaster graminicola* to explore the function of the madreporite. Although he confirmed many of the findings of earlier workers, he observed that the body weight of this species diminished when its madreporite was obstructed. Further, a fluorescently labeled high-molecular weight dextran tracer put into seawater could be found in the body fluids of the animals after a period—a pattern that was largely inhibited when the madreporite was obstructed. Additional study with this tracer method (Ferguson, 1989) established quantitatively that modest amounts of seawater do reach the ambulacral fluid (presumably through the madreporite), but that an even greater volume (nearly 60% of total influx) somehow moves directly into the perivisceral coelomic cavity. Fluorescent microbeads have been used to verify madreporitic inflow in *Leptasterias hexactis*, and point to passage through the Tiedemann's bodies (enigmatic structures on the oral water ring canal) as the probable major route of seawater movement from the madreporite and stone canal to the perivisceral coelomic cavity (Ferguson, 1990b).

From these studies, then, is emerging a new view of the role of the madreporite. It does serve to admit seawater into the starfish body, but this admission is less significant with respect to the inflation of the tube feet, in which osmotic factors predominate, than to fluid replenishment throughout the whole organism and the size control of other fluid compartments. If this is the case, obstruction of the madreporite should have a much more profound effect on volume control (and weight) than on tube foot activity. Such effects might be especially evident in an intertidal species, such as *Pisaster ochraceus*, which appears to experience frequent changes in its fluid volume as a result of tidal stranding, complex postural adjustments, and estuarine salinity variations. These possibilities have been examined in the present study.

Materials and Methods

Work was carried out on specimens of *Pisaster ochraceus*, mostly weighing between 200 and 800 g, collected from rocks at low tide within a few kilometers of the Friday Harbor Laboratories in the San Juan Islands of Washington. The animals were kept in seawater tables for several days before use to ensure their health and stability under laboratory conditions. The seawater temperature remained nearly constant in the short term, between 7 and 9°C, and its osmotic concentration was measured at about 885 mosmol kg^{-1}. During the experiments, the starfish were confined to vessels lined with paper toweling so that they could be removed easily and without damage to their tube feet. The animals were not fed, but they normally eat little during the winter months, when the work was done.

Weight changes of animals were recorded as an indirect measurement of fluid volume variation. The specimens were first blotted dry in a consistent manner with paper toweling for several minutes and then weighed on an electronic balance. As a species adapted to an intertidal existence, *P. ochraceus* took this handling very well, and the wet weights obtained from individual animals were repeatable within 0.20%.

Osmotic measurements were made on some specimens. Small samples of perivisceral coelomic fluid (about 0.5 ml) were withdrawn from these specimens with a syringe and allowed to settle in a capped vial for several minutes. At least three replicates of the samples were analyzed in a Wescor 5500 vapor pressure osmometer, along with ambient seawater for comparison. Studies showed that these measurements were accurate to within 2 mosmol kg^{-1} osmotic difference with 95% confidence.

Because preliminary work had suggested that the effects of madreporite obstruction might take some time to appear, a number of different procedures were explored for sealing this structure without otherwise harming the animals. The approach finally used was quite effective. Commercial hydraulic cement (a mixture of Portland cement and lime) was further triturated to a fine powder with a mortar and pestle. The surface of the madreporite was scraped away with a dissecting needle and the debris blotted up. A small quantity of cement, freshly mixed with a minimum amount of distilled water, was then placed on the wound and worked around with the needle. After the cement had set for several minutes, the animal was returned to seawater, where the plug became fixed firmly in position and quite hard over the next several hours. *Pisaster* tolerated this operation very well, and specimens usually could be kept for up to ten days with the opening still tightly sealed.

Most experiments involved measuring daily changes in the wet weight of six madreporite-obstructed specimens (tests), as well as those of a similar group of unaltered animals (controls). The null hypothesis is that obstructing the madreporite should have no effect on the frequencies of measured daily weight gains and losses; the alternative is that the treatment should prevent weight gains from madreporite fluid uptake and lead to a decrease in weight through gradual fluid losses.

Since the wide range of initial body weights of the specimens available for use could potentially bias many statistical approaches, conclusions were drawn exclusively

from the gain or loss of weight from one measurement to the next. The data were assessed with SPSS-X using a non-parametric two-sample median test. This test evaluated a 2 × 2 contingency matrix of control group and test group daily weight changes above and below a prescribed median of zero. With more than 30 cases, this computes a chi-square statistic and a corresponding probability that the two samples are drawn from populations with the same median. An assumption underlying this approach is that, on any day, a normal undisturbed specimen has an equal chance of showing either a positive or negative weight fluctuation, not materially affected by previous responses. Because a runs test failed to show that observed daily variations of normal animals were statistically predictable, and because these variations were always small relative to the wide ranges of total fluctuations tolerated, this assumption seemed justified.

Four main sets of experiments were undertaken. The first examined the variation of animals kept under stable laboratory conditions for up to 10 days. The second examined responses of animals under similar stable conditions following 12 h of resting on wet paper toweling in air, simulating a prolonged tidal stranding that might leave them dehydrated. The third and fourth sets of experiments examined the fluid volume responses of animals which had been subjected to modest (about 6%) salinity changes that would not be excessively stressful, but comparable to those that might be commonly encountered in their estuarine environment. In these latter experiments, specimens were placed in either hyperosmotic or hypoosmotic media prepared by adding "Instant Ocean" sea salts or distilled water to aquaria of seawater. To monitor changes in the osmotic concentration of the coelomic fluids, we used several additional specimens.

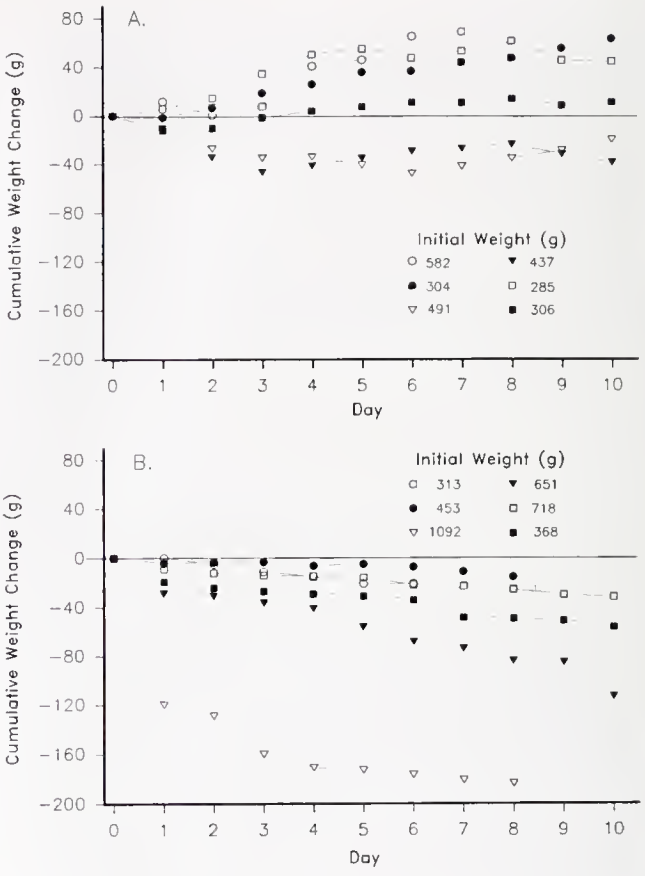

Figure 1. Cumulative change in weight (fluid volume) of specimens of *Pisaster ochraceus* while maintained under stable laboratory conditions for 7 to 10 days. (A) Intact animals (n = 6) have 39 daily periods of increase, 18 of loss. (B) Madreporite-obstructed animals (n = 6) have 2 minor periods of increase, 47 of loss, and 3 no change.

Results

Stable conditions

Groups of control and test animals were monitored daily for weight (fluid volume) changes exhibited in a stable laboratory environment, where neither temperature nor salinity varied beyond detectable limits. The patterns of their cumulative responses may be seen in Figure 1. The weights of individual animals were found to fluctuate spontaneously, over a range of about 20%. These variations appeared to be correlated, at least in part, with postural changes. *Pisaster* is a very "stiff" species, and individuals were observed to hold the same contorted position for many days. When they relaxed and spread out, as they were prone to do after being handled for weighing, their volumes often gradually increased. Within the experimental period of up to 7 to 10 days, every control specimen demonstrated numerous daily intervals of weight gain, as well as instances of daily weight loss. The test animals, on the other hand, showed only 2 minor daily periods of weight increase out of the 52 measurements taken, both within the limits of experimental precision; they had 47 instances of weight loss and three instances of no change. The median test confirmed that the daily responses of the two groups were significantly different ($P < 0.001$). By the final weighing, all 6 test animals had lost weight from the beginning, while 4 of the control animals had gained and 2 had lost weight. In all cases, obstruction of the madreporite had no perceptible effect on the functioning of the tube feet.

Air dehydration

During a 12 h period of exposure to air while resting on moist toweling, both control and test animals lost around 6 to 8% of their body weights (Fig. 2). A good deal of this change appeared to be due to evaporative water loss rather than draining of fluid, as shown by an analysis of the osmotic concentration of the coelomic fluids of

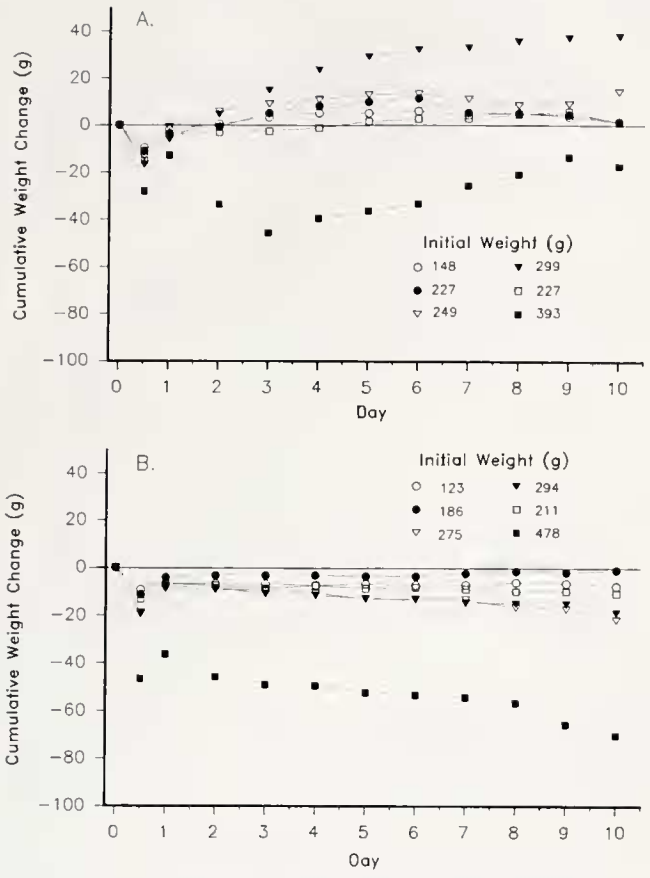

Figure 2. Cumulative change in weight of specimens exposed to the dehydration of a simulated tidal stranding of 12 h and then returned to stable seawater. All regained weight with their re-establishment of osmotic equilibrium after return to seawater. (A) Intact animals (n = 6). (B) Madreporite-obstructed animals (n = 6). After the first day, intact animals (A) show 41 daily periods of gain and 13 loss; test animals (B) 10 minor gains, 39 loss, and 5 no change. The minor gains in the latter group might be due to seal leakage, fluid taken into the stomach, or residual osmotic factors.

four similarly treated normal animals. After 12 h of dehydration, their coelomic fluid osmolality (mean ± S.E.) was elevated 52.1 ± 3.0 mosmol kg^{-1} (5.9%) above ambient seawater, while their weight (mean ± S.E.) had dropped 11.9 ± 2.7 g (6.8%). After 12 h of reimmersion in seawater, their coelomic fluid osmotic level had equilibrated to within 1.1 ± 0.1 mosmol kg^{-1} (0.2%) of ambient seawater, but their weight was still 9.0 ± 2.7 g (5.1%) below the initial mean. Thus, they had gained some water in recovery, but had mainly lost osmolytes. All the control animals quickly assumed normal behavior once they were back in seawater. They then showed 41 daily intervals of weight gain and 13 of loss. The test group (Fig. 2b), like the control animals, also rapidly recovered some weight in seawater, in correspondence with the period of osmotic readjustment. Thereafter, while the control animals gained and fluctuated in weight daily, the test group mostly lost weight: 39 instances to 10 minor gains and 5 instances of no change. The episodes of minor weight gain were observed in 2 of the 6 test animals and could have been caused by leakage around the madreporite seals, fluid taken into the stomach, or residual osmotic factors. Again, a median test confirmed a highly significant difference ($P < 0.001$) between the daily weight change patterns of the two groups. At the end of the experiment, 5 of the 6 control animals showed an overall weight gain, while all 6 tests specimens had lost weight.

Hyperosmotic conditions

Both control (n = 5) and test (n = 6) animals rapidly lost weight for the first 3 to 6 h after being subjected to an osmotic increase in their media from 878 to 935 mosmol kg^{-1}, a change of 6.5% (Fig. 3). By 6 h, both groups appeared to have reached substantial osmotic equilibrium, as monitored by coelomic fluid samples taken from sim-

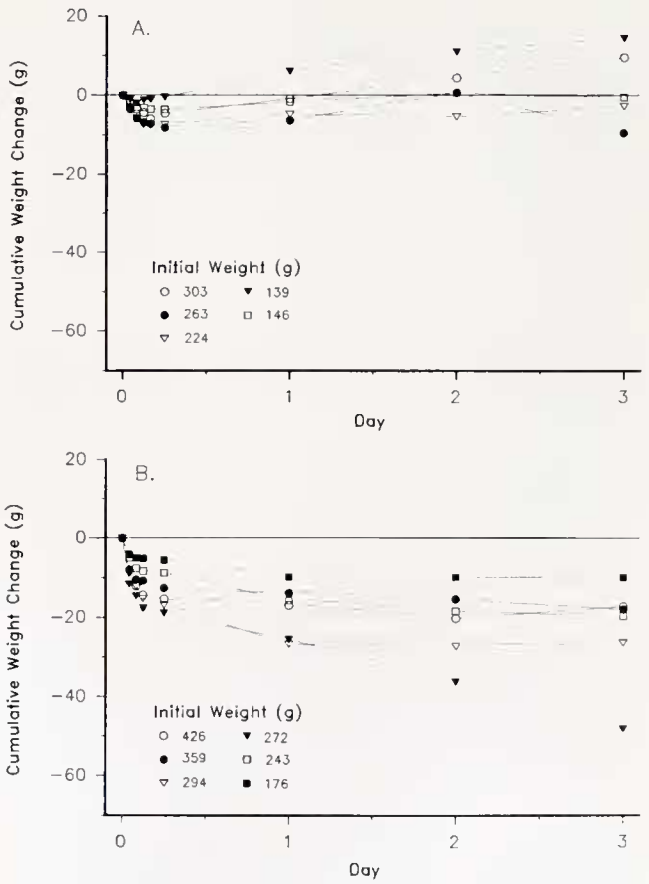

Figure 3. Cumulative weight responses of animals to an osmotic increase in their medium from 878 to 935 mosmol kg^{-1} (6.5%). (A) Intact specimens (n = 5). (B) Madreporite-obstructed specimens (n = 6). Both groups lose fluid over the first few hours while osmotically adjusting. Following the 6 h mark, intact animals (A) show 12 intervals of gain and 3 loss; test animals (B) show 2 gain, 14 loss, and 2 no change.

ilarly treated animals (Fig. 4). Following this period of osmotic adjustment, all of the control animals regained weight for the next 18 h (Fig. 3a). Over the next two days they showed seven instances of weight gain and three of loss. In contrast, all the test animals (Fig. 3b) continued to lose weight for the 18 h following the initial period of adjustment. Thereafter, they had 8 instances of loss, 2 of gain and 2 of no change. The differences in these patterns (after the 6 h adjustment period) were highly significant ($P < 0.001$) by median test analysis.

Hypoosmotic conditions

When exposed to a 6.2% osmotic decrease in the medium, from 885 to 830 mosmol kg^{-1}, 4 of the 6 animals in both the control and test groups temporarily gained weight in the first 4 h, apparently as a manifestation of the osmotic fluxes taking place (Fig. 5). As with the previous experiment, measurements of the coelomic fluids of comparable animals showed that osmotic adjustments of both groups were nearly complete within 4 to 6 h (Fig. 6). For the 18 h after this period, 5 of the 6 control animals lost weight (Fig. 5a). The next day there were three losses and three gains, and the following day, all losses. The test group, on the other hand, showed only losses, except for one instance of no change (Fig. 5b). The transfer to a hypoosmotic medium may have been more stressful to the animals than a transfer in the other direction. In any case, the control animals recorded too few episodes of weight gain, and thus do not show that they responded in a significantly different way from the test animals (median test: $P = 0.112$).

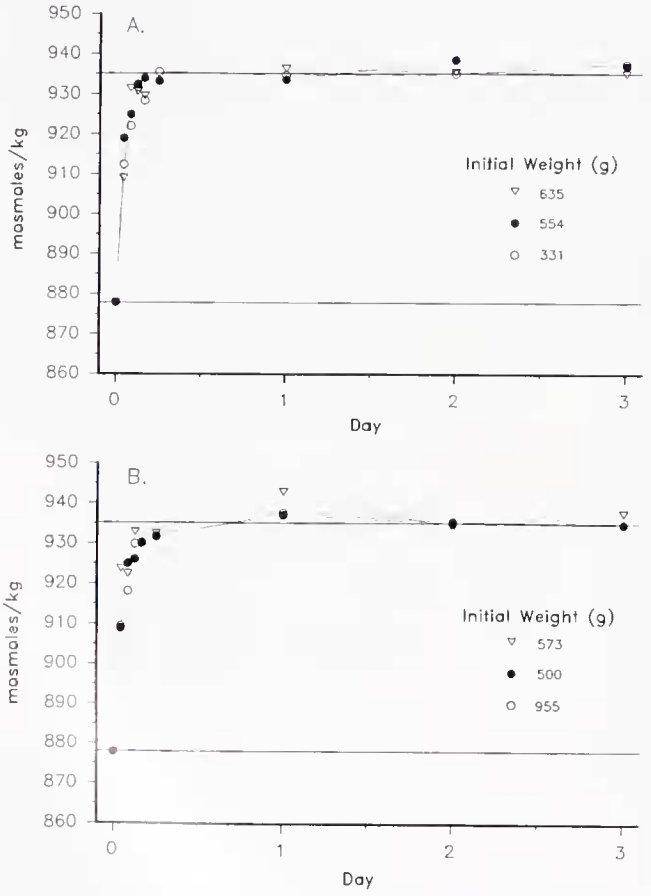

Figure 4. Osmotic changes in the perivisceral coelomic fluid of animals exposed to an increase in their medium from 878 to 935 mosmol kg^{-1}. (A) Intact animals (n = 3). (B) Madreporite-obstructed animals (n = 3). There is little obvious difference in the responses of the two groups, both of which have substantially completed osmotic adjustments by 6 h.

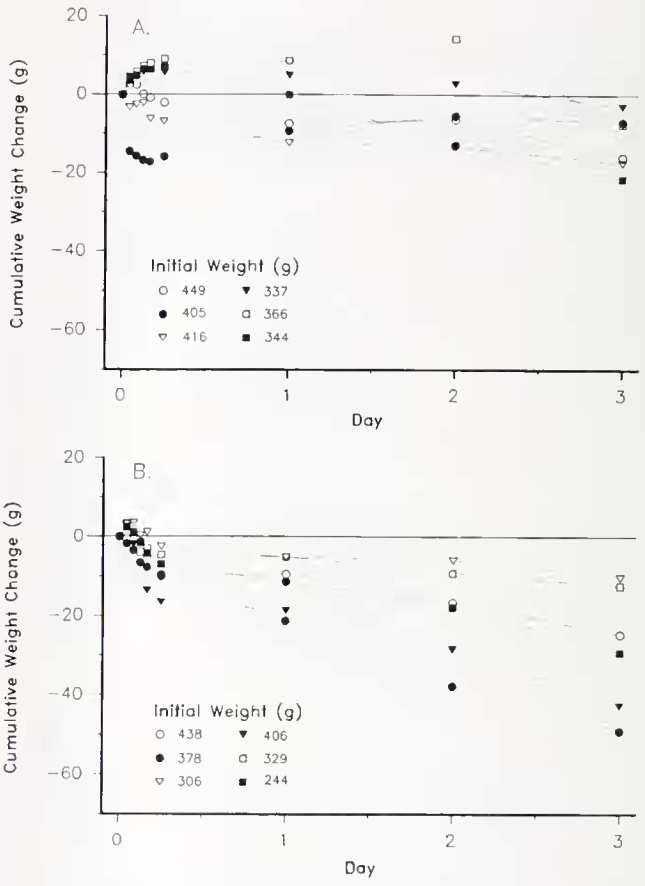

Figure 5. Cumulative weight responses of animals to an osmotic decrease in their medium from 885 to 830 mosmol kg^{-1} (6.2%). (A) Intact specimens (n = 6). (B) Madreporite-obstructed specimens (n = 6). Individuals in both groups show some initial swelling. Following the 6 h mark, intact animals (A) have 4 intervals of gain and 10 loss; test animals (B) show loss in all instances except 1 no change.

Discussion

All four sets of experiments demonstrated markedly reduced capacity of madreporite-obstructed specimens of *Pisaster ochraceus* to retain their fluid volume, even

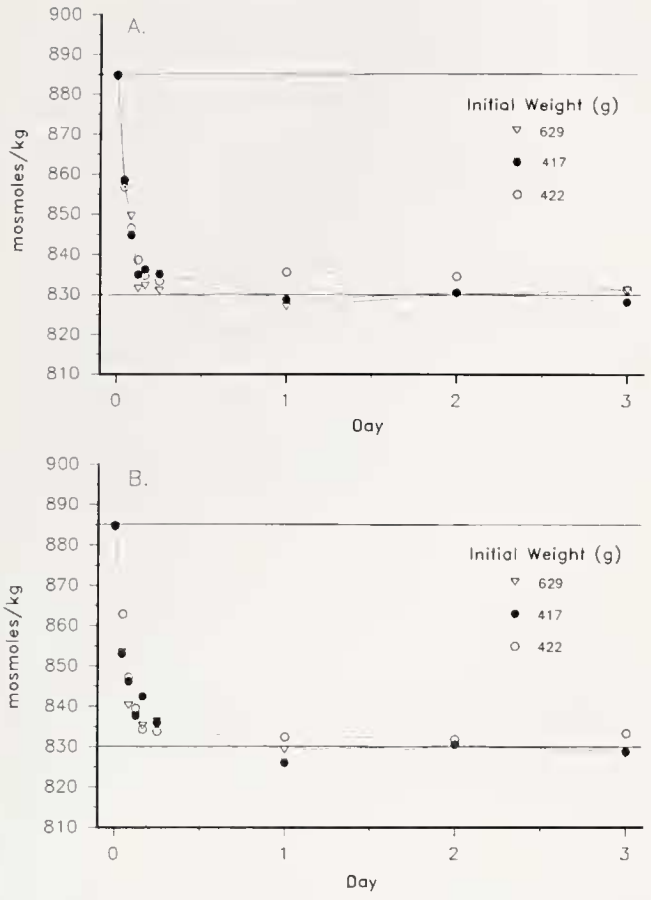

Figure 6. Osmotic changes in the perivisceral coelomic fluid of animals exposed to an osmotic decrease in their medium from 885 to 830 mosmol kg^{-1}. (A) Intact specimens (n = 3). (B) Madreporite-obstructed specimens (n = 3). There is little obvious difference in the responses of the two groups, both of which have substantially completed osmotic adjustments by 6 h.

though the activity of their tube feet appeared undiminished. In fact, every single test specimen weighed less at the end of the study than it did at the beginning. On the other hand, control groups were able to exhibit normal weight fluctuations throughout, except following hypoosmotic stress. Every control specimen showed a period of weight gain, and at the end of the study, 11 weighed more than they did at the start and 12 less (including the hypoosmotic group). Not surprisingly, statistical comparisons by the median test confirmed a highly significant difference between the control and test groups (except following hypoosmotic stress). Thus, these observations strongly support the alternative initial hypothesis: that obstruction of the madreporite should substantially prevent animals from gaining weight and from retaining their body fluid. Although some differences may exist among other asteroid species, and certainly between echinoderm classes, with their different anatomical arrangements, slow but steady inflow of seawater through the madreporite is undoubtedly a basic adaptation for fluid volume maintenance in *P. ochraceus* and probably other starfish. This seawater influx appears to provide a general replenishment of the body fluids, probably the flushing of excretory products, and homeostatic response to changing needs, especially those brought on by alteration in posture, stranding, or fluid losses occurring with osmotic adjustment. The madreporite probably also contributes some water to the tube feet (Ferguson, 1987, 1989, 1990b), although those structures can be kept inflated by the osmotic mechanism previously described and perhaps by fluid diversion through the axial sinus pore (Ferguson, 1991).

The responses shown here by normal animals to salinity changes (as well as osmotic elevation resulting from dehydration) were as might be expected from previous work, reviewed by Binyon (1966, 1972), Diehl (1986), and Stickle and Diehl (1986). After transfer to either hyper- or hypoosmotic medium, rapid exchange of ions and water takes place across the integument, leading to near osmotic equilibrium within a few hours. Binyon (1961, 1964, 1976a,b, 1980, 1984), in his work with *Asterias rubens*, found that the integument was quite permeable to both ions and water, although not necessarily equally. Ellington and Lawrence (1974) and Diehl and Lawrence (1984) similarly demonstrated rapid, but uneven, flux of ions and water in *Luidia clathrata* during hypoosmotic adjustment, leading to some volume increase, which took several days to abate. Shumway (1977) apparently saw such a response in *A. rubens, Solaster pappossus, Henricia sanguinolenta,* and *Astropecten irregularis*, as did Ferguson (1987) in *E. graminicola*. In the major previous work on *P. ochraceus*, however, Stickle and Ahokas (1974) indicate that osmotic adjustment of body fluid during a simulated tidal cycle over a 20‰ salinity range lagged behind ambient seawater and never reached completion. They further stated that there was "little change" in percentage of body water during the cycle. Their graphical data, however, do seem to show some such change, so perhaps their different judgment was only a matter of perspective and the different intentions of the experiments. In any case, the animals in the present study, when transferred directly to the equivalent of about a 1.9‰ salinity change, rapidly reached near osmotic equilibrium with their altered environments, but not such rapid volume stability. The presence or absence of a functional madreporite made relatively little difference in osmotic adjustment, but the madreporite played a significant role in subsequent readjustments of body volume after transfer to a hyperosmotic medium that produced a rapid fluid loss.

By comparing the rates of weight loss by madreporite-obstructed animals to the rates of weight gain by normal specimens, the present data can be used to make specific

estimates of the rate of flow of seawater into the madreporite of *P. ochraceus*. The maximum difference between the control and test groups might be expected to occur in circumstances where the controls are in apparent need of augmenting their body fluids, while the test animals are unable to do so and are suffering physiological losses. Such a situation existed in the hyperosmotically stressed animals between the time that they had largely completed their osmotic adjustment, at 6 h, and before the control specimens had replenished much of their body fluid, at 24 h. Figure 3 shows that within this period, there is indeed a conspicuous difference between the two groups. The six test animals had a weight loss (mean ± S.E.) of 1.16 ± 0.76 $\mu g\ g^{-1}\ h^{-1}$, whereas the five control specimens had a corresponding weight gain of 1.05 ± 0.90 $\mu g\ g^{-1}\ h^{-1}$. The sum of these, 2.21 $\mu l\ g^{-1}\ h^{-1}$, is an estimate of fluid uptake. That value is in agreement with a madreporite flow rate of 2.27 $\mu l\ g^{-1}\ h^{-1}$ measured in *E. graminicola* by fluorescent tracer methodology (Ferguson, 1989). By similar arithmetic, the most rapid rate of weight increase noted in any of the normal control animals in the present study (Fig. 1) corresponds to a net gain of 2.60 $\mu l\ g^{-1}\ h^{-1}$, a value that seems to be well within the reasonable capability of their system.

How seawater taken up reaches the perivisceral coelomic cavity may in part be revealed by other recent work, including some in which fluorescent microbeads were used as a tracer (Ferguson, 1990b). The model developing is that seawater is drawn in through the madreporite by strong currents generated in the ciliated stone canal. This seawater mixes with recirculated fluid from the axial sinus, which also opens into the upper end of the stone canal. The result is a high pressure composite fluid that is pumped into the ring canal. From there, the composite fluid appears to be filtered through the Tiedemann's bodies and tube feet ampullae, counteracting fluid loss from the perivisceral coelom to the higher osmotic concentration in the tube feet.

Other factors also may be involved in body fluid maintenance. A persistent slight osmotic elevation exists in the perivisceral coelomic fluid of most species, although in *P. ochraceus* it is almost negligible [0.47 ± 0.59 mosmol kg^{-1} (± S.E.)] (Ferguson, 1990a). In forms with a more flaccid integument, such as *Pycnopodia helianthoides*, osmotically elevated coelomic fluid [2.18 ± 0.53 mosmol kg^{-1} (± S.E.)] may be of more substantial importance (Ferguson, 1990a). Further studies of the volume responses of *P. helianthoides* would be particularly interesting, but unfortunately, preliminary work has shown that it does not tolerate the present experimental approach. Bulk fluid movement through the digestive systems of starfish might also influence fluid volume relationships (Ferguson, 1987).

How much regulatory control starfish actually have over their fluid volume is also open to question. The present study has demonstrated a net tendency for animals to take up seawater through their madreporites and to contribute that seawater to fluid volume maintenance under a variety of conditions. However, the body weights of the starfish fluctuated widely and could not be considered fixed at any point. So there is as yet little evidence of a sensitive central volume regulatory mechanism other than that achieved from the general balance of water uptake and loss from the several largely independent processes of bulk fluid movement, osmotic uptake and adjustment, pressure filtration, degree of exposure of permeable surfaces (especially on the tube feet), and adjustment of compartment size with posture and feeding. Nevertheless, these animals consistently maintain a low hydrostatic pressure in their body cavities. This is probably only 1 or 2 mm of water in magnitude, but it supports the respiratory papulae and allows extrusion of the cardiac stomach during feeding. This pressure seems to be maintained primarily by the contractile force of the body wall musculature and connective tissue. These animals may be using muscle tone to regulate a low fluid pressure, while allowing their volume to range rather widely. The fluid pressures in these forms are low and so difficult to measure accurately that they have not yet been successfully studied.

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